Table 1. Reports on mRNA of MMPs and TIMPs and Immunohistological Localization of MMPs and TIMPs in Human HCC

Authors	Year	Subjects	MMPs and TIMPs Observed	Ref. No.
Northern Blotting				
Yamamoto *et al.*	1997	30 HCC	MMP-2, -7, MT1-MMP	36
Immunohistochemistry				
Grigioni *et al.*	1991	30 HCC	type IV collagenase	32
Terada *et al.*	1996	11 CCS/6 HCC	MMP-1, -2, -3, -9, TIMP-1, -2	33
Gao *et al.*	2006	72 HCC/6 N	MMP-1,-2, -3, -7, -9, TIMP-1, -2, -3	49
Altadill *et al.*	2009	30 HCC	MMP-1, -2, -3, -7, -9, -11, -13, MT1-MMP (Tissue Microarray)	50
Tretiakova *et al.*	2009	43 HCC/20 N/10 A	MMP-1, -2, -3, -7, -9, E-cadherin (Tissue Microarray)	51
Northern Blotting and Immunohistochemistry				
Arii *et al.*	1996	23 HCC	MMP-2, -9, TIMP-1	34
In Situ *Hybridization and Immunohistochemistry*				
Nakatsukasa *et al.*	1996	21 HCC	TIMP-1, -2	35
Musso *et al.*	1997	6 HCC/7 meta./4 N	MMP-2, TIMP-2	37
Okazaki *et al.*	1997	7 early/7 advanced HCC	MMP-1	22
Harada *et al.*	1998	25 HCC	MMP-2, MT1-MMP	38
Theret *et al.*	1998	22 HCC/41 others	MMP-2, MT1-MMP, MT2-MMP, TIMP-2	39
Rivas *et al.*	1998	40 HCC	MMP-12	40
Ogata *et al.*	1999	37 HCC	MMP-2, MT1-MMP	42
Ashida *et al.*	2000	27 HCC	MMP-9	43
Maatta *et al.*	2000	36 HCC/35 panc, ca.	MMP-2, -9, MT1-MMp	45
Ishii *et al.*	2003	30 HCC	MMP-2, -3, -7, -9, MT1-MMP, MT2-MMP	48
RT-PCR				
Yamamoto *et al.*	1999	30 HCC	MMP-2, -7, -9, MT1-MMP, TIMP-1, -2	41
McKenna *et al.*	2002	7 HCC/8N	MMP-2, -7, -9, TIMP-1,-2	46
Giannelli *et al.*	2002		MMP-2, TIMP-2	47
RT-PCR and Immunohistochemistry				
Sakamoto *et al.*	2000	37 HCC	MMP-1, -2, -7, -9, MT1-MMP	44

MMP and MMP-2 in both hepatoma cells and stromal cells in the invading border of tumor nests. On the other hand Musso *et al.* [37] did not observe positive staining in hepatoma cells, but observed cells positive for MMP-2 mRNA(+)/TIMP-2 mRNA(+)/anti-α-smooth muscle actin(+) (αSMA) which were seemed to be stellate cells at the invasion front. This discrepancy was resolved by Ogata *et al.* [42].

Ogata *et al.* [42] observed MT1-MMP and MMP-2 in hepatoma cells and stromal cells, and both enzymes were detected in the same hepatoma cells. Moreover, both enzymes were associated with tumor dedifferentiation. That is, both enzymes were detected strongly in all cases of poorly differentiated HCC, in 73% of moderately differentiated HCC, but MMP-2 was not detected in well-differentiated HCC. MT1-MMP and MMP-2 mRNA were strongly expressed in the cytoplasm of hepatoma cells as well as stromal cells surrounded by ECM in moderately and poorly differentiated HCCs [42].

The results of *in situ* hybridization by Maatta *et al.* [45] were similar to those by Ogata *et al.* [42]. Hepatoma cells were the main producers of MT1-MMP mRNA, and only a low level of expression could be detected in stromal cells [45]. The expression level in hepatoma cells vary from negative to strongly positive and had a tendency to be associated with poorly differentiated HCC [45]. On the other hand, the expression of MMP-2 mRNA was seen mainly in stromal fibroblasts and endothelial cells and only to a lesser extent in hepatoma cells [45]. It is very interesting that, although MMP-2 was abundantly synthesized by stromal cells, it immunolocalized mainly to tumor cells in HCC [45].

The activation of MMP-2 is related with the coordinated high expression of TIMP-2, and MT1-MMP, but hepatocytes may also modulate the activation of MMP-2 through the expression of MT2-MMP [39].

Hepatoma cells with mRNA expression of both MT1-MMP and MMP-2 are thought to participate in the stromal invasion of HCC, portal invasion, and intrahepatic metastasis. On the other hand, higher expression of MT1-MMP and MMP-2 in non-tumorous cirrhotic liver parenchyma than in tumorous tissue may be associated with inflammatory or post-inflammatory conditions such as chronic hepatitis or liver cirrhosis [37, 39].

MMP-7: Yamamoto *et al.* [36] reported the secretion of MMP-7 protein from human HCC tissue using Western blotting and zymography, but did not present immunohistological staining data.

MMP-1: None of advanced HCC cases showed no MMP-1 mRNA expression [22].

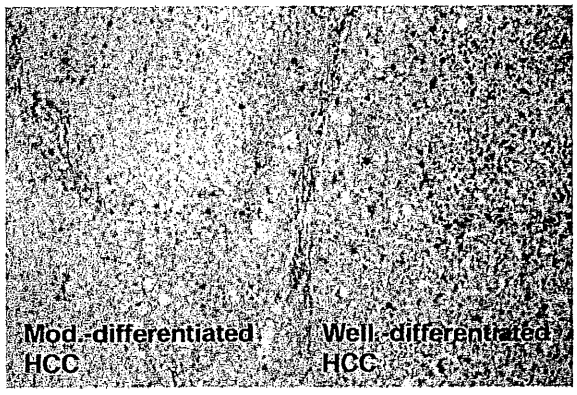

Fig. (2). *In situ* hybridization of early HCC using 45-bp synthetic antisense oligonucleotide probes. Positive grains scattered in well-differentiated cancer lesions. Well, well-differentiated HCC; Mod, moderately differentiated HCC. See the originals appearing in *Hepatology* (1997, 25, 581), which showed the sense figure (control) and magnificated figure for the positive grains. Reprinted with permission.

MMP-12 (human macrophage metalloproteinase): This enzyme not only degrades elastin and a broad range of matrix/non-matrix substrates, but also participates in generation of angiostatin, an internal fragment of plasminogen with an angiogenesis-inhibiting function. *In situ* hybridization revealed MMP-12 mRNA detected in 25 of 40 HCC samples. Patients without positive findings did not produce angiostatin and demonstrated poorer survival than those with positive findings [40]. There was no relationship between the grades of HCC differentiation and positive staining [40].

3.3. MMPs in Early Stages of HCC

Well-differentiated cancer cells in early HCC are known to invade portal tracts and/or fibrous bands resulting in the disappearance of these fibrous tissues [26-29].

MMP-9: Sakamoto *et al.* [44] used semi-quantative RT-PCR in order to investigate the mRNA expression of both MMP-2 and MMP-9 in 37 pairs of HCC and adjunct non-tumor tissue specimens, and confirmed that MMP-9 overexpression was correlated with growth of small HCC.

MT1-MMP and MMP-2: Ogata *et al.* [42] used samples of well-differentiated HCC smaller than 10mm in diameter obtained by ultrasound-guided fine-needle biopsy, and found MT1-MMP detected in one of the six well-differentiated HCCs, but MMP-2 was not detected in any of these same samples.

MMP-1: The authors hypothesized that the degradation of ECM by MMP-1 might be involved in the process of cancer cell invasion. Thus, we investigated the localization of both mRNA and protein of MMP-1 by *in situ* hybridization and immunehistochemical staining, respectively, in 7 cases of early HCC smaller than 2 cm in diameter, and compared with those in 7 cases of advanced HCC [22].

Four of 7 cases with early HCC showed only well-differentiated cancer cells, 2 cases showed both well-differentiated and moderately differentiated cancer cells, the remaining case showed only moderately differentiated cancer cells. *In situ* hybridization revealed that 3 of 4 cases with only well-differentiated HCC expressed MMP-1, 2 cases with both well-differentiated and moderately differentiated HCC showed positive staining (Fig. (**2**)), and one case with only moderately differentiated cells showed negative staining. No case of advanced HCC showed MMP-1 mRNA. The positive cells were well-differentiated cancer cells located at the invading front of the cancer. An interesting finding was that positive cells were scattered in a ratio of approximately 1% to 2 % in cancer. This was very different from the findings of MMP-9 expression reported in other studies described above. Hepatocytes of non-cancerous liver did not express transcripts of MMP-1.

Positive staining of MMP-1 protein was seen in early HCC with well-differentiated hepatoma cells which invaded portal tract (Fig. (**3A**). In another case with early HCC, well-differentiated hepatoma cells positive for MMP-1 protein were compressed by moderately differentiated cancer cells which were negative for MMP-1 staining (Fig. (**3B**)).

Sakamoto *et al.* [44] showed that MMP-1 mRNA expression was significantly higher in tumorous tissue than in non-tumorous tissue, and MMP-1 protein was more strongly expressed than MMP-9 protein immunohistochemically in early HCC.

MMP-1 mRNA was seen in hepatoma cells infiltrated into the small portal tract left in tumor nodule as well as in the fibrous bands surrounding the cancer cell nodule [22]. Very few macrophages or fibroblasts showed MMP-1 mRNA, and no positive cells were

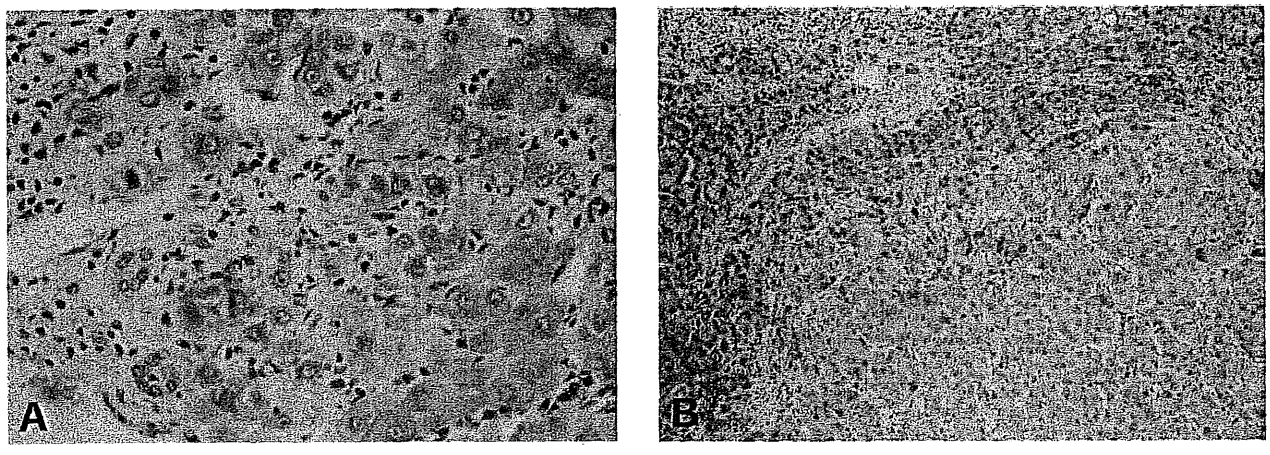

Fig. (3). Immunolocalization of MMP-1 in early HCC. Positive staining was observed in the well-differentiated cancer cells but not in the moderately and/or poorly differentiated cancer cells. (**A**) Well-differentiated cancer cells express MMP-1 protein and invade portal tract. (**B**) Well-differentiated cancer cells expressing MMP-1 protein were compressed by moderately differentiated hepatoma cells. From the original (*Hepatology*, 1997, 25, 582). Reprinted with permission.

observed in non-tumorous tissue [22]. An immunohistochemical study using tissue microarrays revealed that MMP-1 expressed by stromal cells is related to a poorer prognosis [50]. This suggests that MMP-1 may also be an important factor in HCC tumor progression. MMP-1 expression was associated with growth of small HCC in which well-differentiated cancer cells invade the portal tract and fibrous bands, and these fibrous tissues disappear by the participation of MMP-1 [22].

3.4. TIMPs in HCC

Nakatsukasa *et al.* [35] reported that TIMP-1 mRNA and TIMP-2 mRNA in cancerous tissue were homogeneously stained more intensively than in non-tumorous tissue of HCC by *in situ* hybridization. All HCC tissues contained transcripts of both TIMP-1 and TIMP-2 with stronger expression in hepatoma cells than in the surrounding tissue. The expression and distribution of the transcripts for TIMP-1 and TIMP-2 did not differ among the various cancer differentiation stages. The intensity of TIMPs mRNA expression varied from nodule to nodule. Stromal cells in and surrounding the HCC expressed both TIMP-1 and TIMP-2 mRNA, and the expression in stromal cells present in the capsule was especially strong. Immunohistochemical observation of TIMP-1 protein was the same as the mRNA expression finding observed by *in situ* hybridization [35].

On the other hand Musso *et al.* [37] did not observe either mRNA of MMP-2 or TIMP-2 in hepatoma cells, but observed them in αSMA-positive cells at the invasive front. The MMP-2(+)/TIMP-2(+)/αSMA (+) stellate-shaped cells in the perisinusoidal space adjacent to liver tumors are considered as hepatic stellate cells [37].

This discrepancy is probably due to the histological differencesin HCC used in the study above. TIMPs act to modulate the matrix/tumor interaction [41, 46, 47, 49]. Furthermore, TIMPs may play an important role in cell growth, and pro-MMP-2 may be activated by MT1-MMP and TIMP-2 on the cell surface of hepatoma cells, stromal cells and stellate cells resulting in stromal invasion.

TIMP-1 and TIMP-2 expression by stromal cells was associated with a poorer prognosis of HCC as revealed by an immunohistochemical study using tissue microarrays [50, 51].

3.5. Hypotheses of MMPs Expression of Hepatoma Cells

The above observations on MMP-1, -2, -9, MT-1 MMP and TIMP-2 are summarized in Table **2**, which lead to the following hypotheses (Fig. (**4**)):

1. A conversion from adenomatous hyperplasia to atypical adenomatous hyperplasia within liver cirrhosis obtains a phenotype to express MMP-1, resulting in the formation of a new clone. Well-differentiated hepatoma cells proliferate slowly with the ability of stromal invasion [22, 25-29].
2. New clones can proliferate and invade portal tracts and fibrous tissue. Subsequently other clones arise to degrade effectively not only fibrous tissue but also basement membrane. These clones of next generation express MT1-MMP in the process of cancer development.
3. Several well-differentiated hepatoma cells express MT1-MMP and gradually small amounts of MMP-2 and MMP-9, probably stimulated by inflammatory cytokines or TGF-β, which may participate in the stromal invasion or the formation of the thick capsule of cancer nodules. Pro-MMP-9 is activated by MMP-2, or well to moderately differentiated hepatoma cells obtain a phenotype expressing MMP-9. TIMP-1 and TIMP-2 gene transcripts in hepatoma cells are increased by increased expression of MMP-2 and/or MMP-9.
4. MMP-1-positive clones (well-differentiated cancer cells) are compressed by new clones (moderately differentiated cancer cells) and subsequently disappear.

The authors do not have direct evidence for these hypotheses. However, we have previously reported that stem cells derived from bone marrow changed their phenotypes of MMP-13 (a major interstitial collagenase in rodents with a homology to human MMP-1) to MMP-9 expression in the recovery phase from experimental liver fibrosis and cirrhosis [17]. A similar switch of MMP expression may occur in cancer stem cells during hepatic carcinogenesis (Fig. (**4**)).

On the other hand positive staining for both MMP-2 and MMP-9 proteins were observed in inflammatory cells, fibroblasts and endothelial cells. MMP-9 mRNA was detected in mesenchymal cells inside and outside cancer nodules, and in fibrous capsules around the necrosis of cancer nodules [43]. Positive cells for MMP-2 and MMP-9 were numerous and stained strongly for both mRNA and protein in mesenchymal cells and inflammatory cells compared with cells positive for MMP-1 [22, 37, 39]. These positive cells may participate in the degradation of the fibrous tissue to make hepatoma cells easily invade or to form the thick capsule around the nodule (Fig. (**4**)).

4. REGULATORY MECHANISM OF MMPs AND TIMPs IN HCC

The expression of MMPs is mainly controlled at the transcriptional level. The promoter regions of MMPs contain several common elements where the common transcription factors such as AP-1, Ets, and/or NF-κB bind [reviewed in 55].

The authors has clarified that the c-Jun NH2-terminal kinase (JNK) pathway is involved in constitutive MMP-1 expression in a well-differentiated cell line (HLE cells) among 5 HCC cell lines derived from various differentiation stages. c-Jun is phosphorylated by JNK, one of the 4 distinctly regulated MAPK pathways; the other 3 pathways are extracellular signal-related kinases (ERK)-1/2, p38 proteins and ERK5. HLE cells constitutively expressed MMP-1 gene and protein as well as its enzymatic activity without any stimulators such as phorbol ester. MMP-1 gene transcription was under control of the activation of c-Jun through the JNK pathway [23].

Table 2. Positivity of MMPs mRNA and TIMPs mRNA in Hepatoma Cells and Mesenchymal Cells by Early HCC (Less Than 2 cm in Diameter) and Advanced HCC

	Early HCC Cancer Cells/Mesenchym	Reference (s)	Advanced HCC Cancer Cell/Mesenchym	Reference (s)
MMP-1	+/+	22, 44	−/−	22
MMP-2	−/−	42, 45	+/+	42, 45, 48-50
MT1-MMP	±/±	42	+/+	42, 45, 48-50
MMP-9	−/−	43, 45	+/+	43, 45
TIMP-2	−/−	45	+/+	45

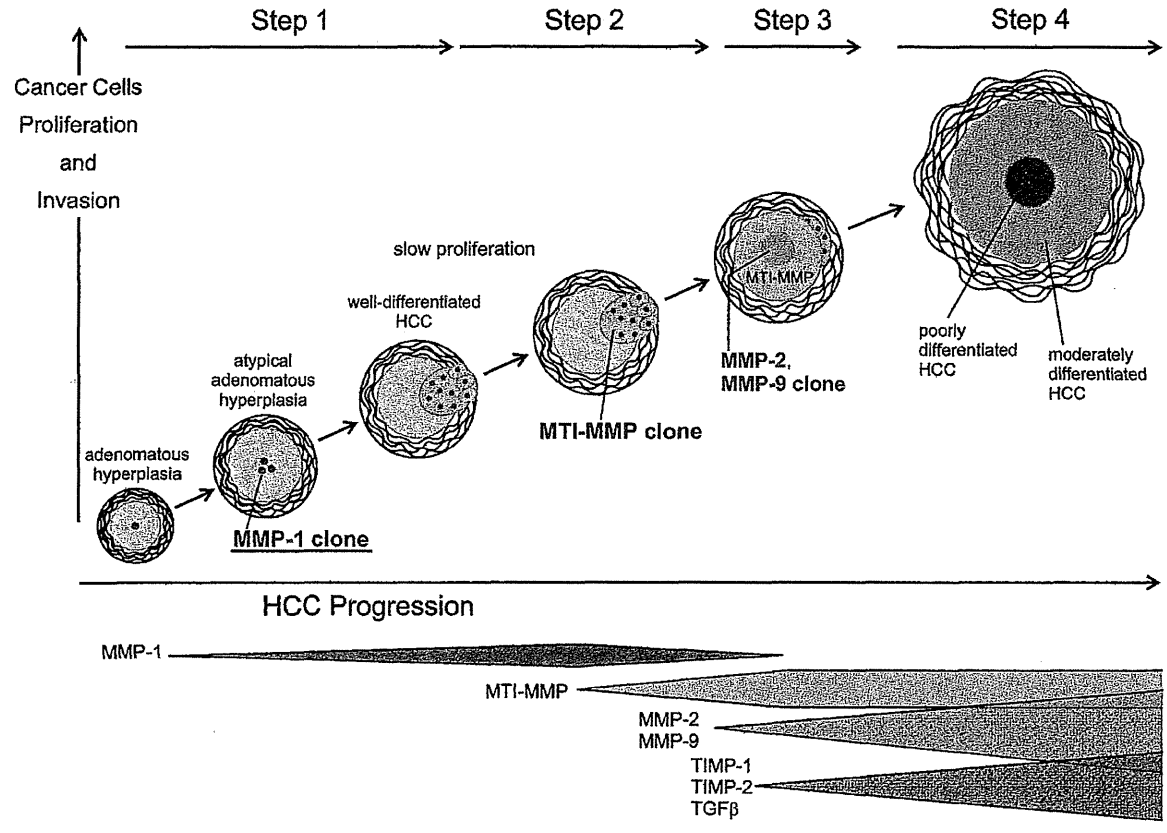

Fig. (4). Hypothesis of MMPs expression related with HCC Progression. Step 1 to 4 are relevant to the number of subsequent MMPs expression in cancer progression wriiten in the hypothesis.

Several anti-cancer agents inhibited expression of MMPs at the mRNA and protein levels in a dose-dependent manner [56-60]. For example, Ide *et al.* [56] reported that menatetrenone (a vitamin K2 analogue) inhibits MMP-1, -3 and -7 expression by suppressing NF-κB and MAP kinase activity. Vitamin K2 inhibits the growth of HCC cells by suppressing cyclin D1 expression through inhibition of NF-κB activation. The NF-κB activity is required for the induction of multiple MMPs even when the promoter regions of some MMPs do not contain an apparent NF-κB binding site [56].

Stefanou *et al.* [57] reported that leptin-induced up-regulation of human telomerase reversed transcriptase (hTERT), and telomerase activity was mediated through binding of STAT3 and Myc/Max/Mad network proteins on hTERT promoter in HepG2 cells, and leptin could affect the progression and invasion of HCC through its interaction with cytokines and MMPs in the tumorigenic microenvironment. In this study it is noted that leptin decreased MMP-1 levels and increased MMP-13 and MMP-9 levels in a dose- and time- dependent manner.

The CD 147 gene in hepatoma cells regulates the expression of multidrug resistant 1 (MDR1), MMP-2 and MMP-9 *via* the ERK1/2-dependent pathway [58]. Glypican-3, a member of the glypican family of cell surface heparin sulfate proteoglycans, is highly expressed in HCC cells and stimulates their growth by up-regulating autocrine/paracrine canonical Wnt signaling, resulting in the expression of MMP-2 and MT1-MMP [59]. Insulin receptor-mediated signaling promotes MMP-2 and MMP-9 expression [60]. On the other hand *Kiss*-1 gene, a putative metastasis suppressor gene, is also reported in HCC related with decreased expression of MMP-9 [61]. PTEN inhibits the migration and invasion of HCC by the down-regulating of MMP-2 and MMP-9 in a PI3K/Akt/MMP-dependent manner [62].

Lysophosphatidic acid (LPA) produced extracellularly by autotaxin (ATX), increases cell survival, angiogenesis, invasion and metastasis [63]. Park *et al.* [63] revealed that ATX transcripts and LPA receptor type 1 (LPA1) protein are elevated in HCC compared with normal tissues. Silencing or pharmacological inhibition of LPA1 significantly attenuated LPA-induced MMP-9 expression and HCC cell invasion.They also found that MMP-9 is downstream of LPA1. Moreover, inhibition of phosphoinositide-3 kinase (PI3K) signaling or dominant negative mutants of protein kinase C and p38 mitogen-activated protein kinase (MAPK) abrogated LPA-induced MMP-9 expression and subsequent invasion [63]. Increased MMP-2, -9 and VEGF caused by hypoxia (*via* ERK1/2) is suppressed with Na^+/H^+ exchanger 1 (NHE1) inhibited by 5-(N-ethyl-N-isopropyl) amiloride [64].

MMPs can be influenced by reactive oxygen species (ROS), resulting in the activation of neutrophils and macrophages at the tumor site with inflammation. These oxidants initially activate MMPs *via* oxidation of the pro-domain cysteine or *via* modification of amino acids of the catalytic domain by hydrochlorous acid in combination with myeloperoxidase [reviewed in 65].

The methylation status of cytokines in CpG dinucleotides located in the MMP promoter also plays a role in controlling gene expression of MMPs, especially in cancer cells. Recent studies on DNA methylase inhibitors, such as 5-aza-2'deoxycytidine, showed a possible induction of hypomethylation of MMPs genes at the promoter level in human hepatoma cells [66].

Overexpressing $p28^{GANK}$ [67] and Fascin-1 [68] are involved in epithelial-mesenchymal transition (EMT) and increase in invasiveness and angiogenesis. $p28^{GANK}$ is an oncoprotein, and up-regulation of $p28^{GANK}$ correlates with cell cycle progression in hepatocytes. The $p28^{GANK}$ activates PI3K/akt/HIF1α to promote TWIST1, VEGF and MMP-2 expression [67]. Fascin-1, an actin bundling protein, is thought to act as a migration factor associated with EMT in HCC and to facilitate the invasiveness in combination with MMPs [68].

Besides well-known cytokines such as VEGF, ILs and others, the inhibitory mechanism of adiponectin was reported. Adiponectin inhibited not only MMP-9 expression but also ROCK/IP10/VEGF signaling pathway resulting in suppression of tumor angiogenesis and cell migration [69]. Thus, the new reports on anti-cancer agents reveal novel regulation mechanisms of MMPs in HCC.

As for the regulatory mechanism of TIMPs there have been very few reports except for the famous paper published by Khokha's group [70]. They showed that TIMP-1 over expression significantly inhibited cellular proliferation and angiogenesis in oncogene (simian virus 40 T-antigen)-induced hepatic carcinogenesis. Although the expression of TIMP-3, a tumor suppressor, has been reported [71], we do not know the regulatory mechanism of TIMPs in HCC.

5. ANTI-CANCER AGENTS FOR HCC AND THEIR REGULATORY MECHANISM

Translational research studies on anti-cancer agents are listed in Table **3**. The authors divided them into three groups, *in vivo* studies, *in vivo* and *in vitro* studies and *in vitro* studies, according to their experimental designs.

The first group includes three candidate anti-cancer agents which were administered to rats with dimethylazobenzene (DAB)- or diethylnitrosamine (DEN)-induced HCC [66, 74, 77]. Murugan *et al.* [66] reported that the dietary administration of DAB induced well-differentiated HCC with increased expression of MMP-2 and MMP-9, and decreased TIMP-2 as well as "reversion-inducing

Table 3. Reported Anti-cancer Agents Involving MMPs Research in HCC

Agent	Origin	MMPs TIMPs Mechanism	Hepatocarcinogenesis in used Animals and/or Hepatoma Cell Lines	Reference
1. *In vivo* studies				
Polyphenon-B	Black tea	Decreased MMP-2, -9, increased TIMP-2, increased RECK HIF1α VEGF VEGFR1 HDAC-1	Dimethylazobenzene (DAB)-induced liver cancer in male Sprague-Dawley Mice	Murugan *et al.* [66]
Morin	Flavonoid	Decreased MMP2, -9, down regulation of COX-2 NF-κB-p65	Diethylnitrosamine (DEN)-induced hepatocarcinogenesis in Wister albino rats	Sivaramakrishnan *et al.* [74]
Bacoside A	Triterpenoid saponin	Decreased MMP-2, -9 protein by immunoblot. and activity	DEN-induced hepatocarcinogenesis in Wister albino rats	Janami *et al.* [77]
2. *In vivo* and *in vitro* studies				
Adiponectin		Decreased MMP-9, ROCK/IP10/VEGF	MHCC97L, Huh7, Hep3B, PLC Male athymic nude mice	Man *et al.* [69]
Pterostilbene	a natural dimethylated analogue of resveratrol	Inhibited MMP (mRNA, protein, actvity) activated by TPA treatment, through ERK1/2, PI3-K/AKT, PKC, NF-κB and AP-1	HepG2 cells and nude mice	Pan *et al.* [73]
OPN ASO		Inhibited MMP-2 and u-PA	HCCLM6 cells, nude mice	Chen *et al.* [81]
Lipocalin 2		Inhibited MMP-2 *via* JNK and PI3K/Akt	Chang liver and Sk-Hep 1 HCC cell lines nude mice	Lee *et al.* [82]
rhFNHN29 and rhFNHC36		Reduced MMP-9 activity and decreased Intgrin αV, β3 and β1	MHCC97H cells, nude mice	Tang *et al.* [83]
3. *in vitro* studies				
menatetrenone	A vitamin K2 analogue	Decreased MMP-1/-3/-7, NF-κB and MAP kinase activity	HepG2, Huh7, Hep3B, HLE cell lines	Ide *et al.* [56]
Leptin		Decreased MMP-1, increased MMP-13/9 *via* human telomerase reverse transcription	HepG2	Stefanou *et al.* [57]
5-(N-ethyl-N-isopropyl) amiloride	Medicinal plant	Decreased MMP-2/-9, VEGF *via* ERK 1/2, inhibition of Na+/ H+ exchanger 1	HepG2 (under hypoxic condition) cell line	Yang *et al.* [64]
Chrysanthemum indicum ethanlic extract	Flavonoid	Decreased MMP-2/-9, increased TIMP-1/-2	MHCC97H (hepatoma) cell line	Wang *et al.* [72]
Hesperidine		Inhibited MMP-9 mRNA *via* NF-κB and AP-1, by inhibitory phosphorylation of p38 kinase and JNK	HepG2 treated with TPA	Lee *et al.* [75]
Hispolon	Mushroom	Decreased MMP-2/-9 *via* ERK ½, PI3K/Akt/FAK	SK-Hep 1 cells	Huang *et al.* [76]
siRNA-targeting BMP-2		Down regulation of MMP-9 and PTEN	SMMC7721 cells	Wu *et al.* [78]
Isofraxidin	Herb	Inhibited MMP-7 *via* ERK1/2, NF-κB, IκB	HuH-7 and HepG2 treated with TPA	Yamazaki & Tokiwa [79]
miR 21		Down regulation of MMP-2/-9 *via* MAPK/ERK	Sk-Hep-1, SNU-182, Hep-G2	Meng *et al.* [80]

cysteine rich protein with Kazal motifs (RECK)" as invasion markers. It also increased hypoxia inducible factor 1α (HIF-1α), VEGF, VEGF receptor 1 and histone deacetylase-1 (HDAC-1) as angiogenesis markers. Simultaneous administration of polyphenon-B significantly reduced the incidence of DAB-induced hepatoma with the reversed phenomenon of invasion and angiogenesis markers. Two other papers also showed that candidate agents suppressed DEN-induced HCC significantly [74, 77]. The second group of studies presented the regulatory mechanisms of the suppression in both *in vivo* and *in vitro* experiments [69, 73, 81-83], while the last group of studies reported 9 candidate agents and discussed whether they could inhibit migration and invasion of hepatoma cell lines [56, 57, 64, 72, 75, 76, 78-80].

The studies of the third group examined signal transduction very carefully, but *in vivo* studies are necessary as the second step of *in vitro* studies. On the other hand, the studies of the first and second groups are not sufficient, because candidate agents and hepatocarcinogens were administered simultaneously. Therefore it is not yet confirmed whether the agents work truly as anti-cancer agents. However, these reports are very valuable for the development of anti-cancer agents.

Gene manipulation therapies have been reported. Wu *et al.* [78] developed small interfering RNA (siRNA)-targeting bone morphogenic protein (BMP)-2 which has an important role in tumor invasion and metastasis. Meng *et al.* [80] established that microRNA-21 regulates PTEN tumor suppressor gene. Chen *et al.* [81] reported that a 2'-O-methoxyethylribose-modified phosphorothioate antisense oligonucleotides (ASO) was effective to knock-down oseteopontin (OPN) which is involved in HCC progression and metastasis. Lee *et al.* [82] transfected lipocain 2 (Lcn2)-cDNA to Chang liver and SK-Hep1 cells resulting in the inhibition of cell proliferation and invasion. Above 4 therapies involved the down-regulation of MMP-2 or MMP-9 as shown in Table **3**.

Although not shown in Table **3**, Muhlebach *et al.* [84] generated several variants of oncolytic measles virus (MV) with fusion proteins by inserting different MMP substrate motifs at the protease cleavage site. They showed that the corresponding MMP-activating oncolytic MV-MMPA1 virus was strongly restricted on primary human hepatocytes and healthy human liver tissue. More efforts are required for its practical use, but the work is very interesting.

6. FUTURE-ORIENTED STRATEGIES BASED ON MMPs SCIENCE

The early stage of HCC expresses MMP-1 mRNA and protein as described above (Table **2**) [22, 44]. The reported anti-cancer agents target MMP-2, MMP-9 and MT1-MMP except "menatetrenone" [56] and "leptin" [57] (Table **3**). These MMPs except MMP-1 are expressed in advanced HCC as mentioned above (Table **2**).

The authors reported that the JNK pathway is involved in constitutive MMP-1 expression only in well-differentiated HCC but not in less differentiated HCC [23]. Eferl *et al.* [85] demonstrated that the requirement of c-jun was restricted to the early stages of HCC development by antagonizing p53 activity, resulting in suppression of apoptosis. The JNK pathway may be required for the early stages of HCC cells. That is, the activation of MMP-1 makes the cells more invasive and proliferative with the inactivation of p53.

In the dedifferentiation process in HCC, the tumor increases in size, less differentiated hepatoma cells arise within well-differentiated tumors and replace them as described above. The subsequent paper by the authors on malignant pancreatic cells revealed that dedifferentiation stimulated MMP-1 transcription through either JNK [23] or ERK1/2 pathway [24]. Table **3** shows that anti-cancer agents for HCC inhibit MMP-2 and/or MMP-9 transcription by modifying multiple MAPK pathways.

A novel strategy has to be developed for preventing the occurrence of well-differentiated hepatoma cells or MMP-1-positive cancer cells. Chen *et al.* [86] reported that well-differentiated cell lines were separated into subpopulations according to CD133, epithelial cell adhesion molecule (EpCAM) and acetaldehyde dehydrogenase (ALDH) expression. CD133+ cells, but not CD133- cells, had the ability to self-renew, create differentiated progenies, and form tumors [87]. We need to clarify whether MMP-1-positive cancer cells are cancer stem cells among well-differentiated hepatoma cells in the early stages of HCC, and whether budding of cancer stem cells are present in atypical adenomatous hyperplasia and adenomatous hyperplasia that exhibit JNK activation and MMP-1 mRNA expression. Kohga *et al.* [88] reported that CD133+ cells were closely related with MMP-2 and a disintegrin and metalloproteinase (ADAM)-9, but they did not describe the MMP-1 expression. Na *et al.* [89] found that HBV-related human HCC were divided into the CD133-high group and CD133-low group and that the CD133-high group showed significantly higher gene expression of MMP-1 and MMP-2. Ectopic expression of HCV core protein constitutively activates AP-1 *via* JNK [90]. HBV or HCV infection increases hepatic production of hedgehog (Hh) ligands and expands the populations of Hh-responsible cells that promote liver fibrosis and cancer stem cells [91]. Activation of JNK is also implicated in alcoholic steatohepatitis (ASH) and NASH leading to the occurrence of cancer stem cells [reviewed in 92]. Cancer stem cells play a key role in cancer cell growth and progression by expanding and relocating bone marrow-niche cells to the tumor [93]. Thick fibrous bands seen in moderately and/or poorly differentiated HCC may be formed by carcinoma-associated fibroblasts expressing α-SMA [94]. Therefore, the involvement of bone marrowniche cells in the early HCC may be different from that in the advanced HCC.

The authors demonstrated the effect of SP600125 on HLE cells to inhibit the expression of MMP-1 mRNA, protein and its enzymatic activity [23]. Novel anti-cancer agents should be explored from this viewpoint. Since Das *et al.* [94] pointed out that JNK promotes and inhibits tumor development in the DEN model of HCC, this dual action of JNK should be considered in the potential use of JNK as a therapeutic target for drug development and treatment of human cancer. The JNK inhibitor agent should be studied *in vivo* and the expression of MMP-1 mRNA and CD133 should be investigated as target markers in early HCC including atypical adenomatous hyperplasia.

7. CONCLUSIONS

The most important strategy to prevent HCC is recovery not only from chronic hepatitis, but also from liver cirrhosis [reviewed in 18, 52, 95]. Although recovery from liver cirrhosis is possible experimentally and theoretically at present, time is required to apply the research results to the treatment of patients with liver cirrhosis. At the same time further studies are needed to investigate the initiation process from adenomatous hyperplasia or atypical adenomatous hyperplasia to early HCC from the viewpoint of MMP-1 expression in relation to cancer stem cells and the effects of JNK inhibitors.

CONFLICT OF INTERESTS

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank Ms. Yoshie Muroya for the systemic literature search and Ms. Cecilia Hamagami for correcting the manuscript in English.

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Received: September 09, 2011 Revised: September 20, 2011 Accepted: November 22, 2011

肝線維症治療の研究はどこまで進展したか？

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索引用語：肝線維症，コラーゲン，TGF-βシグナル，分子標的治療，再生医学

1 はじめに

肝線維症を引き起こす原因は，肝炎ウイルス感染やアルコールの過剰摂取のほか，メタボリック症候群の肝臓病変としての非アルコール性脂肪肝炎(Non-alcoholic steatohepatitis, NASH)，免疫学的機序や金属の代謝異常，肝うっ血など，多岐にわたる．その原因に関わらず，線維化の進行に伴って肝組織中にはコラーゲンをはじめとする細胞外マトリックスが過剰に沈着し，その終末像である肝硬変症では肝細胞の機能低下に伴う肝不全や血行動態の変化による門脈圧亢進症が引き起こされる．高頻度に合併する肝細胞癌の発生を抑止するうえでも，肝線維化研究は臨床的また基礎的に重要かつ喫緊の研究テーマである．

組織におけるコラーゲン含量は，合成と分解とのバランスの上に成り立っており，その均衡が破綻して相対的な合成優位に傾くと，肝をはじめとする諸臓器の線維化を引き起こす．端的にいってしまえば，臓器線維症治療とはマトリックスの合成系と分解系のバランスの是正にほかならない(図1)．最近の10年余りの間に，肝におけるコラーゲンの主要産生細胞である星細胞の活性化機構に加えて，活性化星細胞以外のコラーゲン産生細胞の存在，肝線維化進展を促進する種々の液性因子が果たす役割や他のシグナルとのクロストークなどについて，多くの知見が集積されてきた[1,2]．しかしながら，臨床の現場に目を向けると，今もって肝線維症に対する特異的かつ効果的な治療薬は存在しない．

本稿では，肝線維症治療がこれまでにも増して重要視されている背景を振り返りながら，これまで臨床で試みられてきた肝線維症治療とその問題点，さらには新たな治療戦略の確立を目指した最近の線維症研究の進展について，筆者らの研究成果を含めて概説したい．

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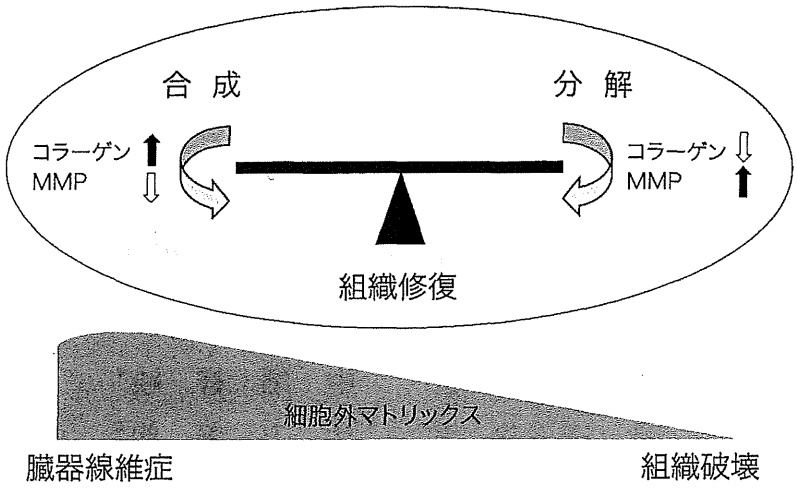

図1　組織のコラーゲン含量と臓器線維症
組織におけるコラーゲン含量は，合成と分解のバランスの上に規定されている．その適切な発現は，組織修復や創傷治癒過程において重要な働きを演じているが，調節機構が破綻をきたすと組織に過剰のコラーゲンが沈着し，諸臓器の線維化を引き起こす．MMP, matrix metalloproteinase (s)．

表1　肝線維化研究が注目されている背景

1. 抗線維化治療を必要とする疾患の増加
2. 肝線維症が可逆的病態であることの認識
3. 肝線維症の非侵襲的診断方法の開発
4. 再生医学・再生医療の進歩

2 今，なぜ線維化研究が注目されているのか？

これまで，とりわけわが国において遅れていた肝線維化研究と線維症治療薬開発の試みが最近にわかに活発になってきたのには，いくつかの理由がある(表1)．まず第1に，C型ならびにB型慢性肝炎に対する治療法の進歩により，近い将来にNASHが慢性肝疾患の主因となることが予想されるなど，わが国における肝臓病診療には大きなパラダイムシフトが起こっている．NASHに対しても体重の減量以外には特異的な治療法が存在しない現状では，早期からの投与により線維化進展を防ぐ薬剤，あるいはすでに肝硬変にまで進行した症例に対して線維の蓄積を改善させて肝細胞機能の回復や肝発癌の抑止をもたらすような薬剤の登場が熱望されている．すなわち，肝線維症はポスト肝炎ウイルス時代の治療ターゲットといえる．

第2に，C型ならびにB型慢性肝炎に対するウイルス治療法の進歩は，肝線維症が可逆的な病態であることを証明した[3,4]．これまで，齧歯類を用いた基礎研究やアルコール性肝線維症・肝硬変症例に対する禁酒指導により日常臨床で感じていた肝線維症の可逆性が実証された意義は大きい．前述したように，組織におけるコラーゲンの含量は合成と分解とのバランスの上に成り立っており，コラーゲンの合成を促進させる原因を取り除くことによって，進行した肝線維症であっても組織学的に改善する．また，原因治療が困難な場合であっても，コラーゲンの合成を抑制する，あるいは分解を適切に誘導することで，肝線維症は治療可能な病態であることがあらためて認識された．

表2　肝線維症に対する抗線維化治療の試み

1)抗線維化効果が認められたもの		
疾患名	治療ないし薬剤名	文献
アルコール性肝硬変	禁酒	5
B型慢性肝炎	ラミブジン	6, 7
C型慢性肝炎	インターフェロン単独療法	3
C型肝硬変	ペグインターフェロン・リバビリン併用療法	4
ヘモクロマトーシス	瀉血療法	8, 9
自己免疫性肝炎	ステロイド，アザチオプリン	10
原発性胆汁性肝硬変	ウルソデオキシコール酸	11
胆管狭窄型慢性膵炎	内視鏡的ドレナージ	12
NASH	ピオグリタゾン	13

2)抗線維化効果が明らかでないもの		
疾患名	治療ないし薬剤名	文献
NASH	グリタゾン製剤，ビタミンE	14, 15
C型慢性肝炎	IL-10	16
(非著効例・再燃例)	インターフェロンγ	17
	グリタゾン製剤	18
	アンギオテンシンII受容体拮抗薬	20

第3には，他稿で詳述されるように肝線維症に対する評価方法の開発があげられる．これまで積極的に検討されてきた血中の線維化マーカーに加えて，超音波装置を用いた肝の弾性度診断が可能になった．肝組織生検は，サンプリング・エラーの可能性はあるものの，今なお最も信頼できる肝線維症の診断手段であるが，全症例に対して治療前後で実施することは現実的でない．弾性度診断もまだ完全とはいえないが，肝線維化治療薬の開発やその臨床治験において，多数の症例の中から比較的均一な対象集団を設定し，比較対照試験により治療効果を判定するうえで，大きな力となることが期待されている．

最後に，近年の再生医学・再生医療の進歩は，肝線維症の病態研究や治療戦略についても大きな知見をもたらしたことを指摘しておきたい．肝の線維化と再生とは常に表裏一体の関係にあり，線維化が進行した肝臓では再生が妨げられ，逆に再生状態にある肝臓は線維化刺激の影響を受けにくい．肝線維化と再生の病態連繋に立脚した新たな線維症治療が模索されており，後述する自家骨髄細胞を用いた肝硬変治療法の開発はその好例である．

3　肝線維症治療薬の現状と問題点

これまで種々の慢性肝疾患に用いられてきた薬剤の抗線維化効果について，主なものを表2にまとめた．このうち，アルコール性肝硬変症例の禁酒[5]，B型慢性肝炎に対するラミブジン治療[6,7]，C型慢性肝炎に対するインターフェロン単独療法[3]もしくは同肝硬変症例に対するペグインターフェロン・リバビリン併用療法[4]など，原因療法が奏効した症例における肝線維症の改善は顕著である．また，ヘモクロマトーシスに対する瀉血療

表3　肝線維化の進展機序からみた線維症治療戦略

1. 星細胞の活性化阻害
2. 活性化星細胞によるコラーゲン産生の抑制
   (1) 遺伝子転写レベル
   (2) 遺伝子転写後レベル
3. 活性化星細胞に対するアポトーシスの誘導
4. Matrix metalloproteinaseによるコラーゲン線維の分解

法[8,9]，自己免疫性肝炎に対するステロイドやアザチオプリンを用いた免疫抑制療法[10]，原発性胆汁性肝硬変症に対するウルソデオキシコール酸治療[11]，胆管狭窄型の慢性膵炎に伴う胆汁うっ滞型肝線維症に対する内視鏡的ドレナージ[12]でも，線維化の進展要因を排除することにより，改善が認められた．一方，現在最も注目を浴びているNASHについては，その病態形成にPPAR-γシグナルもしくは酸化ストレスの関与が指摘されているにも関わらず，ピオグリタゾン投与の有効性を示した一部の報告[13]を除くと，グリタゾン製剤やビタミンE投与の肝線維症に対する抑制効果は概して否定的である[14,15]．

前述したように，C型慢性肝炎に対する抗ウイルス療法の進歩は著しい．今後はプロテアーゼ阻害剤の併用により寛解率は一層高まると考えられるが，重篤な副作用により治療続行が困難な症例や，治療を完遂してもウイルス学的著効が得られなかった症例に対する線維化と発癌の抑制は重要である．これらの非著効例・再燃例に対してIL-10[16]やインターフェロンγ[17]，さらにはグリタゾン製剤[18]の投与が試みられたが，いずれも無効であった．また，アンギオテンシンII受容体拮抗薬ロサルタンの投与が肝組織中の酸化ストレスや線維化関連遺伝子の発現を抑制したことで，その抗線維化効果が期待された[19]が，長期投与では十分な抑制効果は得られなかった[20]．

このように，いわゆる「線維症治療薬」が奏効しない理由としては，薬効自体の問題のみならず，臨床研究デザインの限界があげられる．すなわち，さまざまな経過をたどる多くの慢性肝疾患患者の中から比較的均一な対象集団を設定し，通常10年単位の長期経過をたどる肝線維症に対する薬物の投与効果を，1年前後という短期の比較対照試験で評価することの困難さである．前述したように，感度ならびに特異度に優れた肝線維化の診断方法，しかも線維化の程度(fibrosis)ではなく，ダイナミックな合成系(fibrogenesis)や分解系(fibrolysis)の非侵襲的診断法の開発が期待される．

また，コラーゲンが体内に最も豊富に存在する蛋白質として，組織や臓器の形態保持はもちろん，創傷治癒や組織の修復過程において重要な役割を担っていることから，他臓器へ及ぼす副作用を軽減するには線維化組織あるいはコラーゲン産生細胞特異的に薬剤を運搬・作用させる工夫が必要となる．ビタミンA封入リポソームを用いたコラーゲン特異的シャペロンHSP47 siRNAの星細胞へのターゲティングはその好例であり[21]，さらに最近ではPDGFβ受容体を特異的に認識するペプチドとインターフェロンγを結合させることで活性化星細胞を標的とする線維症治療が動物実験で試みられている[22]．

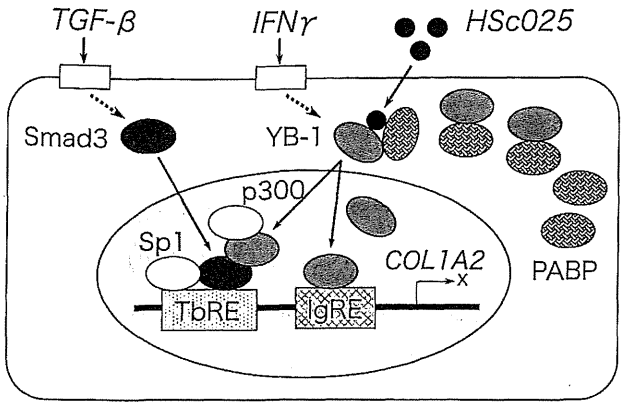

図2　TGF-β/Smadシグナルに拮抗するHSc025の作用機序

YB-1はI型コラーゲン遺伝子(COL1A2)プロモーター上のIFN γ-response element (IgRE)に結合して基礎転写を抑制するとともに，その上流のTGF-β-responsive element (TbRE)に結合するSmad3とCBP/p300 coactivatorとの相互作用を阻害することで，TGF-βによるCOL1A2転写の促進に対して拮抗的にはたらく．新規低分子化合物HSc025は，YB-1とそのanchor proteinであるpoly-A binding protein (PABP)との結合を阻害し，YB-1の核内移行を促進してCOL1A2転写を抑制する．(文献32より，引用・改変)

4　肝線維症に対する治療戦略

活性化星細胞が肝線維化進展において中心的役割を果たしていることから，肝線維症に対する治療戦略を構築することは，とりもなおさず星細胞の活性化機構ならびに活性化星細胞(筋線維芽細胞)によるコラーゲン産生機序の解明にほかならない．肝線維化進展のメカニズムからみた線維症治療のアプローチを表3に示す．それらは，①星細胞の活性化阻害，②活性化星細胞によるコラーゲン産生の抑制，③活性化星細胞に対するアポトーシスの誘導，④Matrix metalloproteinase (MMP)によるコラーゲン線維の分解，に大別される．この際に問題を複雑にしている要因の1つは，星細胞がI型コラーゲンとこれを分解する主要な間質性コラゲナーゼであるMMP-13双方の産生細胞であることである．しかも，MMP-13発現は肝線維症の改善過程においては既存線維を分解するうえで重要であるが，線維化の初期においてはむしろ線維化を進展させるなど，病期によって全く異なる作用を示す．これは，マトリックスの合成と分解とを包括的に，また経時的に解析することの必要性と重要性を示している．

肝線維症に対する幾多の治療戦略の中から，コラーゲン産生において中心的役割を果たすTGF-βの細胞内シグナル伝達物質Smad3を標的とする線維症治療と，新規の線維化改善・再生促進因子を用いた肝硬変に対する細胞治療法について，以下に筆者らの最近の研究成果を紹介したい．

5　TGF-β/Smadシグナルをターゲットとする肝線維症に対する分子標的治療

他稿で詳述されるように，TGF-βは肝線維化の進展過程において中心的役割を担っている．その作用は，星細胞の活性化，コラーゲン遺伝子転写の促進，MMP発現の誘導など多岐にわたる．筆者らもこれまでTGF-βとその細胞内シグナル伝達物質Smadによる I 型コラーゲン遺伝子(COL1A2)の転写調節機構の研究を行い，肝線維化進展におけるその病因的意義を明らかにしてきた[23]．

TGF-βシグナルを標的とする肝線維症に対する実験的治療は，細胞外におけるTGF-βの活性化抑制，細胞膜上の特異的受容体への結合阻害，細胞質から核内へのシグナル伝達の阻止など，それぞれのレベルにおいて試みられてきた．筆者らも，IFN γの細胞内シグナル伝達物質であるYB-1がCOL1A2プロモーター上のTGF-β-responsive elementに結合するSmad3とCBP/p300 coactivatorとの相互作用を阻害

することで，TGF-βによるCOL1A2転写の促進に対して拮抗的に働くことを示した(図2)[24]．さらに，COL1A2エンハンサー・プロモーターを用いてYB-1を過剰発現するアデノウイルスを肝線維症マウスに投与することで，コラーゲン産生細胞特異的に発現誘導したYB-1が線維化進展を抑制することを証明した[25]．最近，これらの知見に基づいて，YB-1とこれを細胞質内に留めるanchor proteinとの結合を阻害し，YB-1の核内移行を促進する新規低分子化合物としてHSc025が開発された(図2)．これを四塩化炭素の反復投与により作製した肝線維症マウスに投与すると，血清トランスアミナーゼ値の有意な低下とともに，肝細胞の脂肪変性と線維の蓄積が有意に改善された[26]．

6 線維肝に対する新規再生促進因子の同定と細胞治療への応用

肝硬変症例に対する自家骨髄細胞の注入療法が国内外の複数の施設で試みられ，一定の成果を上げている[27~29]．線維化の改善とともに肝再生の促進が認められ，比較対照試験の結果が待たれるところである．詳細なメカニズムの解明はこれからだが，骨髄細胞が産生するMMPや増殖因子の関与が指摘されている[30]．肝線維化と再生の病態連繋を考えるうえで，重要かつ興味深い知見である．

筆者らはこれまでに，実験的肝線維症の回復期において骨髄から線維肝組織へと動員された細胞がMMP-13, MMP-9を順次発現することで，線維化改善に寄与することを明らかにした[31]．この際にG-CSFを投与すると，骨髄由来細胞の線維肝組織内への流入と生着が増強し，MMP-9発現の亢進とともに線維化の改善が促進された．G-CSFには肝組織前駆細胞の動員や増殖を促進する作用が知られていることから，G-CSFによる線維肝に対する再生促進機序を考えた．そこで，G-CSFの投与により線維肝組織に浸潤・生着した骨髄由来細胞の遺伝子発現を網羅的に解析し，線維肝の再生を促進する複数の骨髄細胞由来因子を同定した．これらの因子を発現する組換え型レンチウイルスを構築し，これを感染させた骨髄由来間葉系幹細胞を線維肝マウスの脾臓内に投与すると，線維化改善とともに線維肝切除後の肝再生が有意に促進された．現在，その詳細なメカニズムについて解析を行っている．

7 おわりに

現在，「抗線維化剤」としての承認を受けて日常臨床で用いられている薬剤は，ピルフェニドンのみである．それとても，適応は特発性肺線維症に限られ，肝線維症や肝硬変症例に対する使用は適応外であるばかりか，副作用として肝機能障害や黄疸が出現することから，肝機能障害を有する肺線維症患者への使用は慎重投与とされている．培養細胞を用いた*in vitro*試験や動物実験によって多くの抗線維化作用物質が同定・報告されていながら，なぜ臨床で十分な効果を発揮できないのか．どのような患者を対象に選んで，どのような評価系を構築すべきなのか．臨床的にコラーゲン産生細胞特異的に薬剤を到達させて副作用を軽減するには，さらにどのような工夫が必要か．肝臓専門医と創薬研究者に託された課題は大きい．

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今なぜ肝線維化研究が注目されているのか
——序に代えて
Introduction

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これまでわが国において遅れていた肝線維化治療薬の開発に対する産学の関心が最近にわかに高まっているのには，多くの理由がある．まず第1に，ウイルス性慢性肝炎に対する治療法の進歩により，近い将来には非アルコール性脂肪肝炎(non-alcoholic steatohepatitis：NASH)が慢性肝疾患の主因となることが予想されるなど，肝臓病診療には大きなパラダイムシフトが起こっていることである．NASHに対して体重の減量以外には特異的治療法が存在しない現在，早期から線維化進展を防ぐ薬剤，あるいは肝硬変に進行した症例に対して線維の蓄積を改善させて肝細胞機能の回復や肝発癌の抑止をもたらすような薬剤の登場が熱望されている．すなわち，肝線維症はポスト肝炎ウイルス時代の治療ターゲットといえる．第2に，抗ウイルス治療法の進歩は肝線維症が可逆的な病態であることが証明されたことである．これまで，動物実験やアルコール性肝硬変症例に対する禁酒指導により経験していた肝線維症の可逆性が実証された意義は大きい．組織におけるコラーゲン含量は合成と分解とのバランスの上に成り立っており，原因治療が困難であってもコラーゲン合成を抑制する，あるいは分解を適切に誘導することで，肝線維症は治療可能な病態であることがあらためて認識された．第3には，肝線維症に対する非侵襲的評価方法の開発があげられる．血中の線維化マーカーに加えて，超音波装置を用いた肝の弾性度診断が可能になった．肝線維化治療薬の臨床治験において多数の症例のなかから比較的均一な対象集団を設定し，比較対照試験により治療効果を判定するうえで，大きな力となることが期待されている．最後に，近年の再生医学・再生医療の進歩は肝線維症の病態研究や治療戦略においても大きな知見をもたらしたことがあげられる．肝の線維化と再生とはつねに表裏一体の関係にあり，肝線維化と再生の病態連繋に立脚したあらたな線維症治療が模索されている．

このような背景のもとに，本特集においてはすでに一般化しているような教科書的記載はできるだけ避けて，各執筆者自身の研究成果を中心に最近の話題を紹介いただいた．肝線維化研究の進歩，とりわけ臨床応用への展望を理解いただく一助になれば幸いである．

コラーゲン分子種と線維症形成へのかかわり

Contribution of collagen subtypes to fibrillogenesis and fibrosis

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◎コラーゲンは動物の細胞外マトリックスの主成分をなす線維性構造蛋白質である．コラーゲン分子の総量は精妙に調節されているが，さまざまな病的要因により組織中に過多に蓄積される病態が組織・臓器線維症の実体である．分子レベルでみるコラーゲンはさまざまな型が存在し，線維を構築する過程で果たす役割が異なっている．著者らは皮膚創傷治癒過程における新生コラーゲン構築をコラーゲン遺伝子の型別に解析し，そのなかで希少コラーゲン分子種であるV型コラーゲンα3鎖が，コラーゲン線維の会合・集束を抑制することで過剰形成を抑制する働きを見出した．コラーゲンは多細胞組織に必要とされる生育環境の土台でもあり，コラーゲン線維の産生は細胞が生育環境をつくりだそうとする自己防衛反応であるが，線維構築を制御し安定終息させる生体側の仕組みの観点から，機序の解明が期待される．

Key word：線維性コラーゲン，創傷治癒過程，コラーゲンの会合，集束過程，V型コラーゲンα3鎖，線維化の抑制

多細胞生物をレンガ造りの建物にたとえると，細胞がレンガ，細胞外マトリックス(Extracellular Matrix：ECM)はセメントと表現できる．ECMなくしては多細胞構造自体が存在できないので，このたとえはECMの重要性を強調したいときによく用いられている．コラーゲンは動物のECMの主要構成分子であり，総蛋白質の3割を占め，結合組織の支持体として働いているが，それは単なる支持体ではなく，他のECM分子とともに細胞の外部生育環境を構成し“細胞のゆりかご”として機能している．また，細胞に適した“足場”を提供し，情報伝達の媒体となり，細胞に適する居場所や方向性の情報を与え，細胞とともに組織の形態を決定していく能動的な役割をも有しており，かならずしもレンガとセメントのような関係ではない．その詳細な機能については今後の研究に託されている．

本稿では各種のコラーゲン分子のもつ生得的な線維の形成と組織構築の視点から，線維化について述べる．

コラーゲン分子の構造と機能

コラーゲンは現在までに29種の型に分類されるスーパーファミリーを形成しており，46のα鎖遺伝子が確認されている(図1-A)．一般にコラーゲンとしてとらえられているのは線維性コラーゲンであり(Ⅰ，Ⅱ，Ⅲ，V型など7種)，このグループのみがコラーゲン線維を形成する[1]．コラーゲン線維とはコラーゲン分子が束状に会合したもので，成熟コラーゲン分子となった後に規則正しく縦列して形成される(図1-B)．線維束には周期性があり，表面には一定間隔の隙間が形成される．線維性コラーゲンは総量の大部分を占め，非線維性コラーゲンはマイナーコラーゲン(「サイドメモ」参照)と呼ばれるものが多い．

線維性コラーゲンのなかでも組織線維化に関係

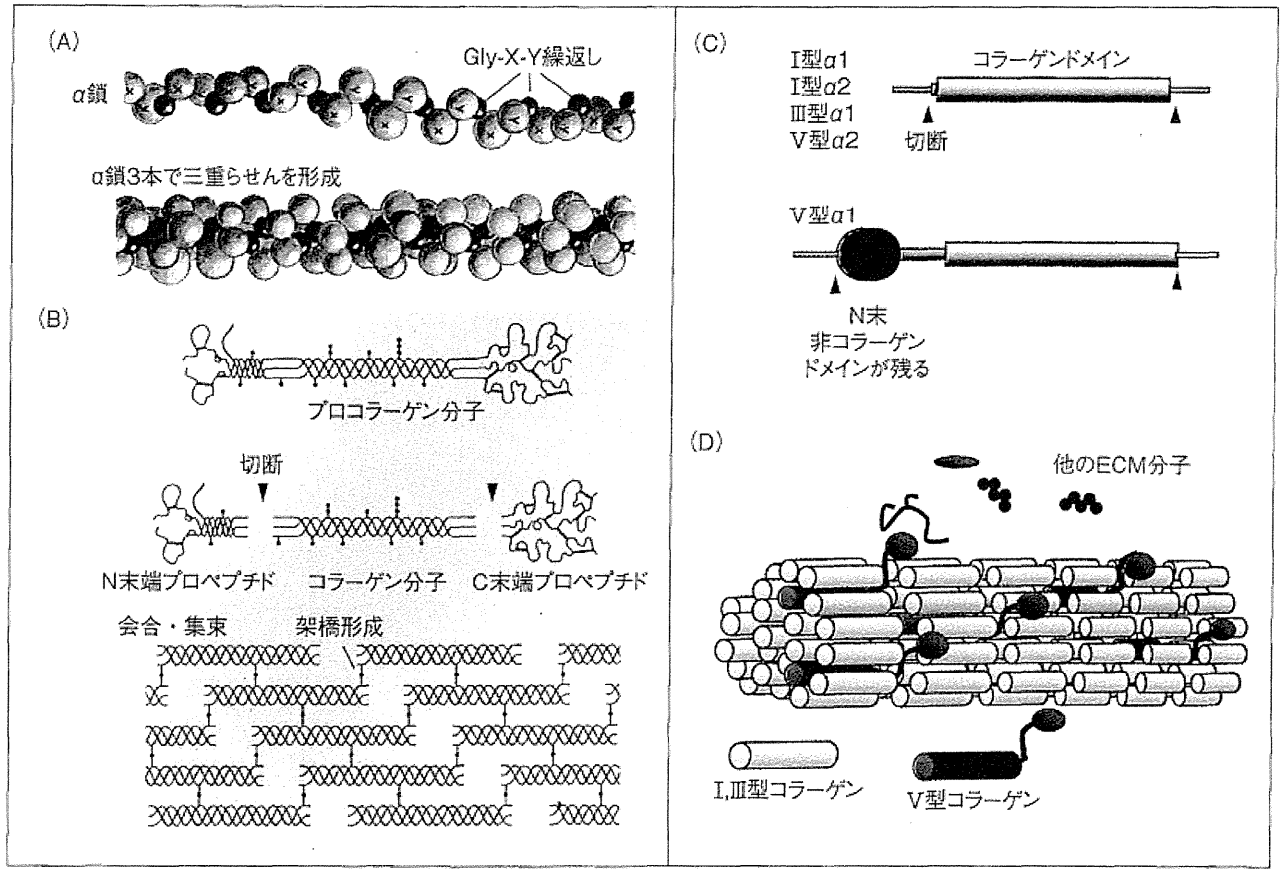

図 1　線維性コラーゲンの構造

A：コラーゲンドメインのアミノ酸一次構造．Gly-X-Y 繰返し構造と，小さいグリシン残基が縒りあわせの中心となり 3 重らせん構造を形成するモデルを示す(Bruce, A. et al.: Molecular Biology of the Cell. Garland Science, 2007 より)．

B：線維性コラーゲンの会合．コラーゲンは分泌直後はプロコラーゲンとして N，C 末のプロペプチドをもつが，細胞外でプロペプチドが切断を受け，成熟コラーゲン分子となり，会合体を形成する．この束が電子顕微鏡で観察されるコラーゲン線維である〔大塚吉兵衛，安孫子宜光著：医歯薬系学生のためのビジュアル生化学・分子生物学(改訂第 3 版)．日本医事新報社，2008，p.167 より〕．

C：I 型，III 型，V 型コラーゲンの構造の違い．I 型，III 型，V 型 α2 鎖はプロペプチドを除くと純粋な 3 重らせん構造が残るが，V 型 α1 鎖と α3 鎖はプロペプチド以外に N 末に残る非コラーゲンドメイン，テロペプチドを有する構造をしている．

D：線維性コラーゲンの会合模式図．I 型，III 型を基本としてコラーゲンの束が形成される．V 型コラーゲンは N 末のテロペプチドの為，線維の外周部に配置し，他の ECM 分子とのインタラクションを通じて線維の構築を調節するとされている(文献[2]より改変)．

するのは，I，III，V 型コラーゲンであり，I 型はもっとも多い成分で組織線維症の“主犯”としてとらえられている．これに対し，III，V 型は形成途中の細いコラーゲンに多く含まれ，線維化進行のマーカーとなっている．また V 型 α1 鎖は，N 末端にテロペプチドと呼ばれる大きな非コラーゲン領域を有している(図 1-C)．この立体的な特徴のため V 型コラーゲンはコラーゲン重合体の表面に配置され，線維の隙間から N 末端ドメインを突き出す形態をとり(図 1-D)，V 型コラーゲン含量が増すと線維径が細くなる．N 末端領域の役割は解明されていないが，ECM 分子と相互作用しながらコラーゲン線維の径を調節すると考えられている[2]．V 型 α1 鎖の KO マウスはまったくコラーゲン線維を形成できず致死となり，線維の形成初期に核を形成する役割があるとみられる[3]など，線維化抑制について重要なヒントを握っているものと考えられる．

創傷治癒におけるコラーゲン線維の新生

コラーゲン分子の種類と線維構築の側面から線維化がどのように進行していくか，創傷治癒をモ